

Factors in Various Fractions of Meat Homogenates That Affect the Oxidative Stability of Raw Chicken Breast and Beef Loin

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ABSTRACT: The fractions of meat homogenates were analyzed to find the factors that determine the susceptibility of raw chicken breast and beef loin to lipid oxidation. The fractions used in this study were meat homogenate, precipitate, and supernatant of homogenate after centrifugation, and high and low molecular weight fractions from the supernatant. Chicken breast showed greater oxidative stability than beef loin during 10-d storage ($P < 0.05$). All fractions from chicken breast showed lower amounts of free ionic iron and myoglobin and higher total antioxidant capacity (TAC) than those from beef loin during storage. The TAC level of chicken breast maintained during storage. This suggested that the oxidative stability of chicken breast was ascribed to high, stable total antioxidant capacity with low level of catalysts for lipid oxidation. The water-soluble high molecular weight fraction, which contained myoglobin, was responsible for the high lipoxygenase-like activity and lipid oxidation potential (LOP) in beef loin. TAC in all fractions from beef loin decreased during storage. This suggested that high myoglobin content in beef loin caused the imbalance between pro- and antioxidant factors leading to the high susceptibility of beef loin to lipid oxidation. Myoglobin served a major source of catalysts, ferrylmyoglobin, hematin, and/or free ionic iron, for lipid oxidation.

Keywords: lipid oxidation, lipid oxidation potential, myoglobin, total antioxidant capacity (TAC)

Introduction

Lipid oxidation is a major cause of quality deterioration in meat. In addition, lipid oxidation causes loss of nutritional values and generates and accumulates compounds that may pose continual risks to human health (Kanner 1994; Min and Ahn 2005). Muscle has a large number of endogenous prooxidants such as myoglobin and ionic irons, which are essential elements for life. These are tightly regulated by various endogenous antioxidant factors such as reducing compounds (for example, ascorbic acid), natural antioxidants (carnosine, anserine, and α -tocopherol), and antioxidant enzymes (catalase, superoxide dismutase, and so on) in muscle (Chan and Decker 1994; Min and Ahn 2005). The capability of muscle to regulate these prooxidants, however, may be decreased rapidly during the conversion of muscle to meat (Morrissey and others 1998).

Among the meats from different animal species, beef is the most susceptible to lipid oxidation (Rhee and Ziprin 1987; Rhee and others 1996; Kim and others 2002; Min 2006). It has been suggested that the amounts of endogenous catalysts such as myoglobin and free ionic iron, reducing compounds, antioxidants (for example, carnosine and related dipeptides), and catalase are important factors that determine the rate of lipid oxidation in meat (Chan and Decker 1994; Rhee and others 1996; Pradhan and others 2000; Min 2006). Myoglobin can catalyze lipid oxidation in meat via various ways: metmyoglobin can react with hydrogen peroxide or lipid hydroperoxides to produce ferrylmyoglobin and release hematin or free ionic iron, which can catalyze initiation and propagation of

lipid oxidation (Dix and Marnett 1985; Harel and Kanner 1985; Kanner and Harel 1985; Rhee and others 1987; Kanner and others 1988; Kim and Sevanian 1991; Reeder and Wilson 1998; Egawa and others 2000; Baron and others 2002; Baron and Andersen 2002). Therefore, the extent of catalase activity can be a determining factor for the different rates of lipid oxidation in meat (Rhee and others 1996; Pradhan and others 2000). Reducing compounds such as ascorbic acid can serve as either an antioxidant or a prooxidant, depending on its relative concentration to the iron present (Giulivi and Cadenas 1993; Kroger-Ohlson and Skibsted 1997; Halliwell and Gutteridge 1999). Min (2006) showed that the amounts of reducing compounds in meats from different animal species vary significantly. However, differences in the amount of total fat and polyunsaturated fatty acid (PUFA) had little effect on the oxidative stability of raw meat because most lipids are stored in adipose tissues where the amounts of prooxidants are low (Min and Ahn 2005).

Although several studies were conducted to determine the effect of cytosol fractions from meat or fish on the development of lipid oxidation in model systems (Kanner and others 1991; Undeland and others 2003), no attempt has been made to compare the effects of anti- and prooxidant factors in various fractions of meat homogenates from different animal species. The objective of this study was to elucidate why the oxidative susceptibilities between chicken breast and beef loin are different by determining the strengths of antioxidant and prooxidant factors in various fractions of meat homogenates from the 2 meat sources.

Materials and Methods

Chemicals and reagents

Metmyoglobin (from equine skeletal muscle), linoleic acid, 2-thiobarbituric acid (TBA), ferrozine (3-(2-pyridyl)-5,6-bis

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(4-phenyl sulfonic acid)-1,2,4-triazine), neocuproine (2,9-dimethyl-1, 10-phenanthroline), ferric chloride, chelex-100 chelating resin (50 to 100 dry mesh, sodium form), butylated hydroxytoluene (BHT), and Tween-20 were purchased from Sigma (St. Louis, Mo., U.S.A.). Ascorbic acid, cupric chloride, and hydrogen peroxide were obtained from Fisher (Pittsburgh, Pa., U.S.A.). All other chemicals and reagents used were of reagent grade. Deionized distilled water (DDW) by Nanopure infinity™ ultrapure water system with ultraviolet (UV) (Barnstead, Dubuque, Iowa, U.S.A.) was used for the preparation of all reagents and buffers. All DDW and buffers were treated with the Chelex-100 chelating resin to remove any free metal ion before use.

Sample preparation

Beef loins and chicken breast meat were purchased at 4 different local distributors. A beef loin from each distributor was used as a replication and 4 chicken breast meats from each distributor were pooled and used as a replication. Meats for each replication were ground separately through an 8-mm plate twice and 80-g patties were prepared. The patties were individually packaged in oxygen-permeable zipper bags (polyethylene, 4 × 6, 2 mil.; Associate Bag Co., Milwaukee, Wis., U.S.A.) and stored at 4 °C.

Meat homogenate (H) was prepared 0, 5, and 10 d of storage by homogenizing the ground meat with 3 volumes of 50 mM acetate buffer (pH 5.6) using a Brinkman Polytron (Type PT 10/35; Brinkman Instrument Inc., Westbury, N.Y., U.S.A.) for 15 s at speed 7. A portion of the homogenate was stored at 4 °C for subsequent analyses and the rest were centrifuged at 15000 × g for 30 min at 4 °C. After centrifugation, the supernatant was collected and the precipitate was re-suspended in three volumes of 50 mM acetate buffer (pH 5.6) and centrifuged. After repeating the process one more time, the precipitate was suspended with three volumes of the buffer and used as a precipitate (P) fraction. The supernatants collected were pooled, filtered through a Whatman paper No. 1 twice, and used as a supernatant (S) fraction. Half of the supernatant (S) fraction was used to prepare for the high molecular weight (HMW) fraction and the other half for the low molecular weight (LMW) fraction. The HMW fraction was obtained by dialyzing (MW cut-off of 12000; Sigma) the supernatant at 4 °C against 50 volumes of 50 mM acetate buffer (pH 5.6) 3 times. After dialysis, the retentate was recovered and centrifuged at 3000 × g for 40 min at 4 °C. The supernatant was collected and used as a HMW fraction. The LMW fraction was obtained by ultrafiltration of the supernatant using a Centricon Plus-20 centrifugal filter (MW cutoff of 10000; Millipore, Billerica, Mass., U.S.A.). The flow diagram for the sample preparation is shown in Figure 1. H fraction represented ground meat sample because meat is usually ground and homogenized for chemical analysis. S fraction represented the cytosol of muscle cells and included water-soluble anti- and prooxidants such as myoglobin, free ionic iron, low molecular weight reducing compounds such as ascorbic acid, NAD(P)H, glutathione, and so on, and antioxidant enzymes such as catalase and superoxide dismutase. P fraction contains reducing enzyme systems attached to the membrane of mitochondria and microsome and water-insoluble substances such as hemosiderin. S fraction was divided into HMW and LMW fractions to separate myoglobin and free ionic irons.

Nonheme and total iron, myoglobin, metmyoglobin percentage, total antioxidant capacity index, ferric iron reducing capacity, lipoxygenase-like activity, and lipid oxidation potentials for each fraction and meat homogenate were determined. Lipid oxidation of patties was also determined at 0, 5, and 10 d of storage, but total iron content was determined at 0 d only.

Lipid oxidation

Lipid oxidation was determined by the method of Ahn and others (1998). Briefly, 5 g of ground meat were homogenized with 15 mL DDW and 100 μL BHT solution (6% in 100% ethanol) using a polytron for 15 s at speed 7. The meat homogenate (1 mL) was mixed with 2 mL TBA/TCA solution (20 mM TBA/15% trichloroacetic acid (TCA, w/v)). The mixture was incubated in boiling water bath for 15 min. After cooling, the mixture was centrifuged at 3000 × g for 15 min. The absorbance of the supernatant was determined at 531 nm against a reagent blank. The amount of 2-thiobarbituric acid reactive substances (TBARS) was expressed as mg malondialdehyde (MDA) per kg meat.

Total iron, nonheme iron, myoglobin, and percentage metmyoglobin

Total iron content was measured by the combination of the wet-ashing method (Carpenter and Clark 1995) with some modification. Ground meat (approximately 2 g) was accurately weighed into a 125 mL Erlenmeyer flask and mixed with 15 mL concentrated nitric acid. The mixture was placed at room temperature overnight for predigestion. The predigested solution was heated on a hot plate (150 °C) until dry. Three to 4 mL of Caro's acid (50% hydrogen peroxide:concentrated sulfuric acid = 4:1) containing peroxymonosulfuric acid were added to the flask left on a hot plate until all peroxide was evaporated. After cooling, the digest was quantitatively transferred to a 10-mL volumetric flask using 0.01 N HCl as a rinse. The diluted sample (0.5 mL) and 0.01 N HCl (1 mL) were mixed with 0.5 mL of 1% ascorbic acid in 0.2 N HCl (w/v) and left at room temperature for 5 min. Then, 0.8 mL of 30% ammonium acetate (w/v), 0.2 mL of ferrozine color reagent (6.1 mM ferrozine, and 14.4 mM neocuproine in 0.14 N HCl solution) were added to the test tube and thoroughly mixed. After 10 min, the absorbance of the mixture was determined at 562 nm against a reagent blank. The total iron content was expressed as microgram total iron per gram meat.

Nonheme iron content was determined by the ferrozine method of Ahn and others (1993). In brief, sample (1.5 mL) and ascorbic acid (0.5 mL, 1 % in 0.2 N HCl, w/v) were thoroughly mixed with 11.3% TCA solution (w/v, 1 mL). After 5 min at room temperature, the mixture was centrifuged at 3000 × g for 15 min at 20 °C. The supernatant (2 mL) was mixed with 0.8 mL of 10% ammonium acetate

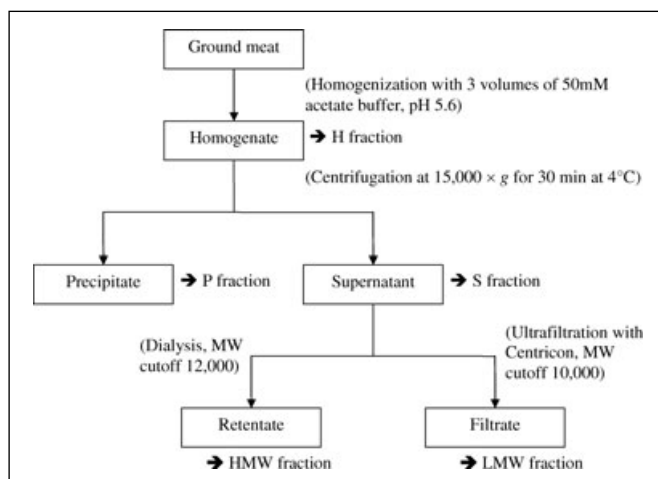


Figure 1 – Flow diagram of fraction preparation from raw chicken breast and beef loin. H = homogenate fraction; P = precipitate fraction; S = supernatant fraction; HMW = high molecular weight fraction from supernatant fraction; LMW = low molecular weight fraction from supernatant fraction; MW = molecular weight.

(w/v) and 0.2 mL of the ferrozine color reagent. After 10 min of color development at 22 °C, the absorbance was determined at 562 nm against a reagent blank. The nonheme iron content in each fraction was expressed as microgram nonheme iron per gram meat.

Myoglobin and metmyoglobin percentage were determined by the method of Krzywicki (1982) with slight modification. Samples were centrifuged again at $15000 \times g$ for 1 min at 4 °C. The absorbance of the supernatant was read at 525, 545, 565, 572, and 730 nm. The contents of myoglobin and metmyoglobin were calculated on the basis of the following equations and expressed as milligram myoglobin per gram meat:

$$\text{Myoglobin concentration (mg/g meat)} = (-0.166R_1 + 0.086R_2 + 0.088R_3 + 0.099) \times A^{525} \times 0.0175 \times \text{dilution factor}$$

$$\text{Metmyoglobin content (\%)} = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100$$

where A^{525} is absorbance at 525 nm, R_1 is A^{572}/A^{525} , R_2 is A^{565}/A^{525} , and R_3 is A^{545}/A^{525} .

Total antioxidant capacity and ferric ion reducing capacity

Total antioxidant capacity (TAC) was determined by the cupric reducing antioxidant capacity (CUPRAC) method of Apak and others (2004). Sample (1.1 mL) was thoroughly mixed with the mixture of 10 mM copper(II) chloride (1 mL), 1.0 M ammonium acetate (1 mL), and 7.5 mM neocuproine in 96% ethanol (1 mL). After standing at room temperature for 1 h, the absorbance at 450 nm was read against a reagent blank. The total antioxidant capacity (TAC) was expressed as microgram ascorbic acid equivalent per gram meat.

Ferric ion reducing capacity (FRC) in each fraction was measured by the method of Kanner and others (1991). Sample (1 mL) was mixed with 1 mM ferric chloride solution and placed for 10 min at room temperature. After the addition of 11.3% TCA solution, the mixture was centrifuged at $10000 \times g$ for 10 min. The supernatant (2 mL) was reacted with 0.8 mL 10% ammonium acetate and 0.2 mL the ferrozine color reagent for 10 min. The absorbance was read at 562 nm against a reagent blank. The ferric ion reducing capacity (FRC) was expressed as microgram ascorbic acid equivalent per gram meat.

Lipoxygenase-like activities

Lipoxygenase-like activities of S, HMW, and LMW fractions were measured by the modification of Gata and others (1996). Linoleic acid (10 mM) in 0.02 N NaOH solution was used as a substrate solution, which was flushed with and kept under nitrogen. Tween-20 was used as an emulsifier. The reaction mixture was composed of 80 μ L of the substrate solution, 80 μ L of each fraction as an enzyme solution, and 50 mM acetate buffer (pH 5.6) to a final volume of 1 mL. Lipoxygenase-like activity was assessed by measuring the absorbance increase at 234 nm due to the generation of conjugated dienes from linoleic acid at 27 °C. The results were expressed as units of activity (U) per gram meat, calculated from the molar extinction coefficient of hydroperoxyl linoleic acid ($\epsilon = 25000$ per M/cm). One unit of lipoxygenase-like activity was defined as the amount of enzyme catalyzing the formation of 1 μ mol of hydroperoxide per minute.

Lipid oxidation potential

Lipid oxidation potential (LOP) of each fraction was determined using a liposome model system prepared with egg phospholipids. An aliquot of phospholipids dissolved in chloroform were transferred to a scintillation vial and evaporated under nitrogen gas to make a thin film on the vial wall. Each fraction was added to the phospholipid-coated vial and then the vial was shaken vigorously for 2 min to make fraction-liposome solution with final concentration of 3 mg phospholipids per milliliter fraction. The fraction-liposomes were incubated at 37 °C for 90 min to accelerate lipid oxidation. Lipid oxidation in the fraction-liposomes was determined at 0, 15, 30, 60, and 90 min. After addition of BHT solution (10 μ L) to stop the reaction, an aliquot (0.5 mL) was mixed with 1 mL of TBA/TCA solution, and, subsequently, was incubated in boiling water bath for 15 min. After cooling, the mixture was centrifuged at $3000 \times g$ for 10 min. The absorbance of the supernatant was determined at 531 nm against a reagent blank. The extent of lipid oxidation in the fraction-liposome was expressed as mmol MDA per kg phospholipids, calculated from the molar extinction coefficient of 1.56×10^5 per M/cm. The phospholipid liposome model system with 50 mM acetate buffer (pH 5.6) was used as a control.

Statistical analysis

Four replications were used for all sample analyses. Data were analyzed using the JMP software (version 5.1.1; SAS Inst. Inc., Cary, N.C., U.S.A.) and reported as means and standard error of means (SEM). Differences among means were assessed by Tukey's method ($P < 0.05$) (Kuehl 2000). In addition, Student's *t*-test was used to compare the difference between the same fractions from chicken breast and beef loin during storage.

Results and Discussion

Lipid oxidation and total iron content in meat

TBARS values of beef loin were significantly higher than those of chicken breast (Figure 2A). TBARS value of beef loin significantly increased during storage, but that of chicken breast did not change. This result confirmed previous studies (Rhee and others 1996; Kim and others 2002; Min 2006), which indicated that chicken breast was less susceptible to oxidative stresses than beef. The concentrations of heme pigments, free ionic iron, antioxidant enzymes such as catalase, and reducing compounds are suggested to be the major factors influencing different susceptibility of beef and chicken breast to lipid oxidation (Rhee and others 1996; Min 2006). The extent of polyunsaturation in total fat was not a determining factor for the different oxidative stability between raw beef and chicken breast (Min 2006). In addition, total iron content in beef loin was much higher than that in chicken breast as expected (Figure 2B).

Myoglobin and nonheme iron in fractions

As expected, the myoglobin concentration in beef loin was greater than that in chicken breast (Table 1). The myoglobin concentrations in both of LMW fractions from chicken breast and beef loin were not shown because those were undetectable. The concentrations of myoglobin and metmyoglobin percentage (Table 1) in all fractions from chicken breast did not change during storage. The myoglobin concentrations in all fractions from chicken breast meat in this study may be overestimated due to the turbidity of the extracts (Kranen and others 1999).

The myoglobin concentrations in H, S, and HMW fractions from beef loin significantly decreased during storage, but increased in precipitate (P) fraction due probably to the deposition of denatured

myoglobin. In addition, metmyoglobin percentage increased significantly in H, S, and HMW fractions from beef loin, but did not change in P fraction. The metmyoglobin percentage in the H fraction at day 10 was significantly lower than those in S and HMW fraction due to the presence of the P fraction. The P fraction including mitochondria and microsomes, which contain many enzymes including metmyoglobin-reducing enzyme system (Rhee 1988; Arihara and others 1995; Thomas 1995), retarded the autoxidation of myoglobin, which is a major source of catalysts/initiator such as ferrylmyoglobin, hemein, and free ionic iron for the development of lipid oxidation (Kim and Sevanian 1991; Baron and Andersen 2002; Min and Ahn 2005). In addition, the metmyoglobin percentage in the HMW fraction increased rapidly, but an increase in the metmyoglobin percentage in the S fraction was delayed at the early stage of storage due to the presence of the LMW fraction including low molecular weight reducing compounds. However, those com-

pounds seemed to be unstable because metmyoglobin percentage in the S fraction increased rapidly at the late stage of storage. It was shown that LMW fractions of cytosol from fish and turkey have inhibitory effect on hemoglobin and myoglobin-catalyzed lipid oxidation due to reducing compounds and/or antioxidants in LMW fraction (Kanner and others 1991; Undeland and others 2003).

Initial (day 0) nonheme iron contents in H, P, S, and HMW fractions from chicken breast was significantly lower than their counterparts from beef loin during storage (Table 1). However, nonheme contents in LMW fractions from both meats were undetectable at days 0 and 5. Nonheme iron contents in the H and P fraction increased significantly during storage, but not in the S fraction. It is assumed that most of nonheme iron generated in chicken breast during storage may be stored in water-insoluble fraction, especially hemosiderin, and thus is not activated for lipid oxidation. Hemosiderin is a water-insoluble complex of iron, other metals and

Table 1 – Myoglobin concentration (mg myoglobin/g meat), percent (%) metmyoglobin, and nonheme iron content (μg nonheme iron/g meat) in various fractions from chicken breast and beef loin during storage at 4 °C.

	Chicken breast					Beef loin					
Storage	H	P	S	HMW	LMW ^A	H	P	S	HMW	LMW ^B	SEM
Myoglobin concentration						mg myoglobin/g meat					
0 d	1.16 ^c	0.35 ^{de}	0.56 ^d	0.25 ^{dey}	—	5.97 ^{ax}	0.39 ^{dey}	3.05 ^{bx}	2.93 ^{bx}	—	0.09
5 d	1.11 ^c	0.35 ^e	0.58 ^{de}	0.42 ^{dex}	—	5.14 ^{ay}	0.71 ^{dx}	2.89 ^{bx}	2.67 ^{bx}	—	0.07
10 d	1.16 ^c	0.40 ^d	0.62 ^d	0.44 ^{dx}	—	4.50 ^{az}	1.00 ^{cx}	2.43 ^{by}	2.26 ^{by}	—	0.06
SEM	0.04	0.02	0.03	0.04	—	0.18	0.08	0.08	0.08	—	—
Percent (%) metmyoglobin ^B						% metmyoglobin					
0 d	62.15 ^b	59.26 ^{bc}	61.22 ^b	46.70 ^{dy}	—	57.71 ^{bcy}	62.65 ^b	51.50 ^{cdz}	71.43 ^{ay}	—	1.70
5 d	64.60 ^b	65.48 ^{bc}	64.44 ^b	60.79 ^{bx}	—	68.39 ^{bx}	66.39 ^b	67.86 ^{by}	95.75 ^{ax}	—	1.78
10 d	62.93 ^c	63.76 ^c	60.22 ^c	59.11 ^{cx}	—	81.97 ^{bx}	65.68 ^c	91.11 ^{ax}	93.92 ^{ax}	—	3.28
SEM	0.47	1.00	0.86	2.14	—	3.63	0.54	3.70	3.34	—	—
Nonheme iron content						μg nonheme iron/g meat					
0 d	1.02 ^{by}	0.42 ^{cdz}	0.32 ^{cd}	0.27 ^d	0.00 ^e	2.06 ^{az}	0.54 ^{cz}	0.52 ^{cdy}	0.50 ^{cdy}	0.00 ^{ay}	0.06
5 d	1.30 ^{by}	0.65 ^{cdy}	0.34 ^{de}	0.23 ^{ef}	0.00 ^f	3.03 ^{ay}	1.24 ^{by}	0.73 ^{cy}	0.62 ^{cdy}	0.00 ^{ely}	0.08
10 d	1.73 ^{bx}	0.81 ^{cx}	0.59 ^{cd}	0.35 ^{cd}	0.04 ^d	4.34 ^{ax}	1.87 ^{bx}	1.60 ^{bx}	0.86 ^{cx}	0.41 ^{cdx}	0.15
SEM	0.09	0.04	0.07	0.02	0.02	0.24	0.12	0.11	0.06	0.04	—

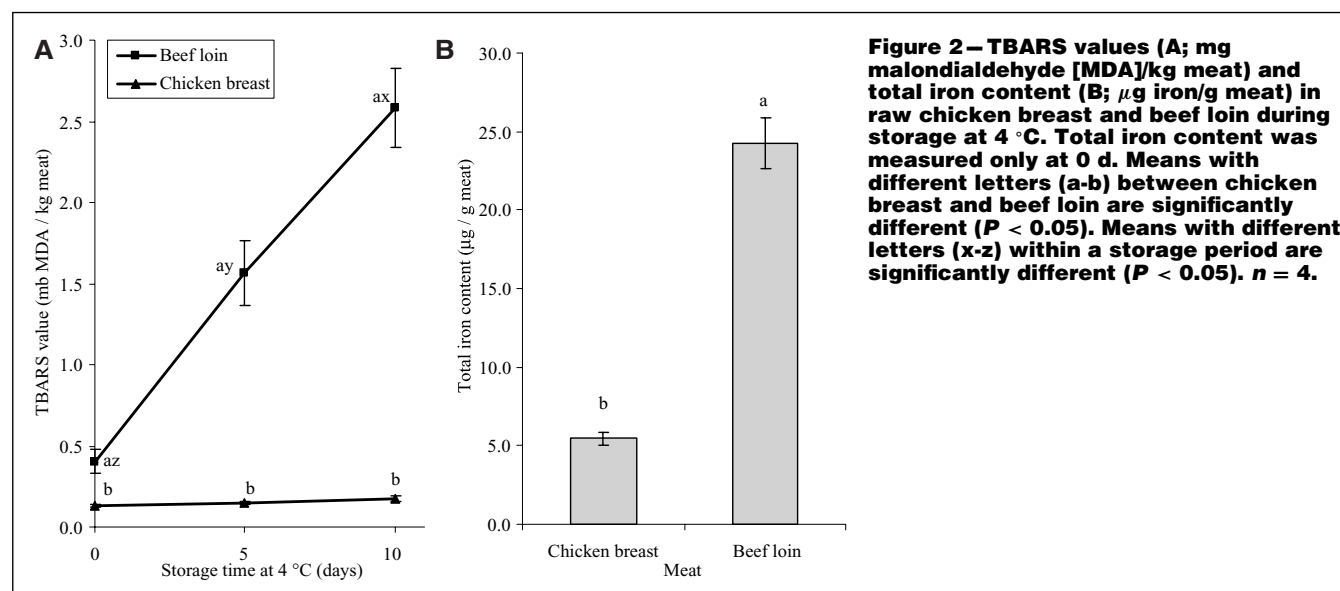
^{a–f}Means with different superscripts within the same row are significantly different ($P < 0.05$). $n = 4$.

^{x–z}Means with different superscripts within the same column are significantly different ($P < 0.05$). SEM = standard error of the mean.

H = homogenate fraction; P = precipitate fraction; S = supernatant fraction; HMW = high molecular weight fraction from supernatant fraction; LMW = low molecular weight fraction from supernatant fraction.

^ABecause myoglobin in LMW fractions from chicken breast and beef loin was not detected, percent metmyoglobin for those fractions were not determined.

^BPercent (%) metmyoglobin: percentage of total myoglobin.



proteins, and considered as a ferritin decomposition or polymerization products (Decker and Hultin 1992).

Nonheme iron content in all fractions from beef loin significantly increased during storage, and the rates of increase in all fractions from beef loin were greatly higher than those from chicken breast (Table 1). Nonheme iron contents in the H and P fractions increased gradually throughout the storage time. However, increases in nonheme iron contents in the S, HMW, and LMW fractions were only observed from day 5 to day 10. The increase in nonheme iron content in LMW fraction was especially great in this period. Therefore, it is assumed that released nonheme irons in the beginning of storage may be captured in ferritin, which is subsequently converted to hemosiderin. However, the amount of nonheme iron not bound to proteins increased in S and LMW fraction from day 5 to day 10 because the amount of ferritin in beef is very limited (Hazell 1982). The major source of nonheme iron in beef is myoglobin and accounts for over 90% of heme proteins present in beef (Hazell 1982). The interaction of metmyoglobin with H_2O_2 or lipid hydroperoxide results in the release of free ionic iron (Harel and others 1988). Free ionic iron can serve as a catalyst in the production of $\cdot OH$ from H_2O_2 as well as in the degradation of lipid hydroperoxides to produce peroxy and alkoxy radicals, which can initiate lipid oxidation and/or be self-degraded to the secondary products of lipid oxidation (Min and Ahn 2005). However, reducing compounds are essential to convert ferric to ferrous ion, a catalyst for Fenton reaction. Ahn and Kim (1998) suggested that the status of ionic iron is more important than the amount of iron.

Total antioxidant capacity (TAC) and ferric ion reducing capacity (FRC)

Total antioxidant capacities (TAC) in H, S, HMW, and LMW fractions from chicken breast during storage were significantly higher than those in their counterparts from beef loin, but those in P fraction from both meats were not different (Table 2). TAC in all fractions from chicken breast did not change during storage, but TAC in those from beef loin decreased significantly. Chicken breast had higher initial TAC and stronger endogenous capability to stabilize TAC than beef loin. In addition, water-insoluble (P) fraction showed significantly higher TAC than water-soluble (S) fraction in both chicken breast and beef loin during storage. Reducing enzyme systems bound to mitochondria and microsome in P fraction may be contributed to higher TAC in P fraction and may have played an important role in maintaining oxidative stability of meat. HMW

fraction had significantly higher TAC than LMW fraction in both meats during storage.

As shown in Table 2, the initial (day 0) ferric ion reducing capacities (FRC) of H, S, and LMW fraction from chicken breast were twice or 3 times as high as those from beef loin, respectively, but rapidly decreased to the same level as those from beef loin at days 5 and 10. The rapid decrease of FRC in S and H fractions from chicken was due to the decrease of FRC in LMW fraction. These observations indicated that chicken breast has large amounts of unstable low molecular weight reducing compounds such as ascorbic acid, NAD(P)H, glutathione, and thiol compounds (Kanner and others 1991; Kanner 1994), which appears to be negligible in beef loin. The initial FRC in P and HMW fractions from chicken breast was not different from that from beef loin, and did not change during storage. After unstable reducing compounds in LMW fraction lose their FRC at the earlier stage of storage, FRC in H, S, and LMW fraction from chicken breast were not different from those of beef loin at days 5 and 10. This indicated that both chicken breast and beef loin had the same levels of stable systems to maintain the FRC during storage. It is assumed that FRC in H fraction from both meats at days 5 and 10 is due mostly to the water-insoluble fraction containing reducing enzyme systems in mitochondria and microsomes, and partly due to the stable compounds in water-soluble fraction. The FRCs in P, S, HMW, and LMW from beef loin decreased gradually during storage. The FRC in P and HMW fractions from beef loin decreased during storage due probably to oxidative damage in enzyme systems and sulfhydryl part of proteins. It is assumed that strong interactions among various reducing components, proteins, and stable low-molecular-weight reducing compounds in P, HMW, and LMW fractions might be contributed to the stability of FRC in H fraction from beef loin during storage.

The free radical scavenging activity of antioxidants such as α -tocopherol is achieved by 1 electron reduction of free radicals, resulting in the termination of lipid oxidation chain reaction. The TAC and FRC used in this study represented the reducing capacity of antioxidants to reduce cupric ($Cu(II)$) and ferric ($Fe(III)$) ions to cuprous ($Cu(I)$) and ferrous ($Fe(II)$) ions, respectively, and expressed as microgram ascorbic acid equivalent per gram meat. Huge differences between TAC and FRC for every fraction from chicken breast and beef loin were observed (Table 2), and those gaps should be primarily attributed to the differences in 1 electron reduction potential of copper ion and iron ion and their reactivity in redox reactions (Buettner 1993; Lynch and Frei 1995; Burkitt 2001; Apak and others 2004). The 1 electron reduction potential

Table 2—Total antioxidant capacity (TAC) and ferric ion reducing capacity (FRC) of various fractions from chicken breast and beef loin during storage at 4 °C. TAC was determined by the cupric reducing antioxidant capacity (CUPRAC) method. TAC and FRC were expressed as μg ascorbic acid equivalent/g meat.

Storage	Chicken breast					Beef loin					SEM
	H	P	S	HMW	LMW	H	P	S	HMW	LMW	
Total antioxidant capacity (TAC)											
0 d	2943 ^a	1307 ^c	651 ^d	469 ^e	109 ^{gh}	2605 ^{bx}	1336 ^{cx}	369 ^{efx}	278 ^{gix}	87 ^{hx}	37
5 d	2755 ^a	1308 ^c	663 ^d	483 ^e	107 ^h	2151 ^{by}	1340 ^{cx}	298 ^{fy}	235 ^{gy}	52 ^y	34
10 d	2763 ^a	1301 ^c	676 ^d	477 ^{de}	104 ^f	1912 ^{by}	1160 ^{cy}	272 ^{ely}	214 ^{ely}	40 ^{fy}	56
SEM	88	72	8	6	3	71	23	9	6	4	
Ferric ion reducing capacity (FRC)											
0 d	24.72 ^{ax}	6.93 ^{cd}	12.33 ^{bx}	2.02 ^e	12.19 ^{bx}	12.86 ^b	7.76 ^{cx}	4.63 ^{dex}	3.69 ^{ex}	6.49 ^{cdx}	0.54
5 d	13.21 ^{ay}	7.69 ^b	5.38 ^{bcy}	2.01 ^d	4.08 ^{cdy}	14.11 ^a	5.44 ^{bcy}	4.38 ^{cdxy}	2.99 ^{cdy}	3.91 ^{cdy}	0.56
10 d	13.90 ^{ay}	6.92 ^b	3.63 ^{cdz}	2.05 ^d	1.66 ^{dz}	12.13 ^a	4.89 ^{bcy}	3.07 ^{cdy}	2.00 ^{dz}	3.03 ^{cdy}	0.51
SEM	1.17	0.65	0.31	0.21	0.33	0.70	0.40	0.33	0.11	0.32	

a–i Means with different superscripts within the same row are significantly different ($P < 0.05$). $n = 4$.

x–z Means with different superscripts within the same column are significantly different ($P < 0.05$). SEM = standard error of the mean.

H = homogenate fraction; P = precipitate fraction; S = supernatant fraction; HMW = high molecular weight fraction from supernatant fraction; LMW = low molecular weight fraction from supernatant fraction.

of Cu(II)/Cu(I) couple (0.615 volt at pH 7 in the presence of neocuproine; Burkitt 2001) is higher than that of Fe(III)/Fe(II) couple (0.11 V; Buettner 1993). Therefore, the substances with their reduction potentials (E°) between 0.11 and 0.65 V are not thermodynamically feasible to reduce Fe(III) to Fe(II), but Cu(II) to Cu(I). Because the reduction potentials of the major free radicals involved in free radical chain reactions of lipid oxidation such as hydroxyl radical (2.31 V), alkoxyl radical ($\text{RO}^\bullet/\text{ROH}$, 1.6 V), and peroxy radical ($\text{ROO}^\bullet/\text{ROH}$, 1.0 V) (Koppenol 1990) are higher than that of copper ion (0.65 V), those are thermodynamically capable of scavenging free radicals and terminate lipid oxidation processes. Furthermore, copper ions are chemically more reactive than iron ions, resulting in faster kinetics than iron ions in redox reactions (Lynch and Frei 1995; Apak and others 2004). For example, the rate constant in Fenton reaction catalyzed by the copper ion would be 61.8 times faster than that by the iron ion (Halliwell and Gutteridge 1999). In addition, Mira and others (2002) reported that most of the flavonoids assessed in their study showed higher reducing capacity for copper ions than iron ions. Consequently, a large part of the TAC in each fraction may not participate in the reduction of Fe(III) to Fe(II) although the FRC is likely to be a part of TAC.

Ascorbic acid, an important biological reducing agent, can serve as an antioxidant or prooxidant in meat, depending upon its relative concentration to free ionic iron content: an antioxidant at higher concentrations and a prooxidant at lower concentrations (Decker and Hultin 1992; Gorelik and Kanner 2001). Therefore, it is assumed that the FRC can work as an antioxidant or prooxidant activity, depending upon the concentration of free ionic iron. The FRC in H fraction from chicken breast and beef loin were similar at days 5 and 10, but TBARS values at days 5 and 10 (Figure 2) were much higher in beef loin than in chicken breast. This indicated that greater increase in free ionic iron in beef loin during storage (Table 1) was partially responsible for the higher TBARS values in beef loin than those in chicken breast.

Lipoxygenase-like activity

Rao and others (1994) suggested that myoglobin has lipoxygenase (LOX)-like activity because it forms ferrylmyoglobin after interacting with H_2O_2 . It is known that ferrylmyoglobin has an ability to initiate lipid oxidation by abstracting hydrogen atom from bisallylic carbon on fatty acid chain to produce lipid hydroperoxides (Kanner and Harel 1985; Rhee and others 1987; Chan and others 1997; Baron and Andersen 2002). The LOX-like activity of myoglobin in linoleic acid emulsion system was 8.39 units per mg myoglobin.

S and HMW fractions from beef loin showed significantly higher LOX-like activities than LMW fraction due to myoglobin in S and HMW fractions (Table 3). LOX-like activities in S and HMW fractions increased during storage. However, the changes of LOX-like

activities in S and HMW fractions during storage were highly correlated with the metmyoglobin percentage ($r = 0.90$ and 0.94 , respectively) and TAC ($r = -0.83$ and -0.88 , respectively) in both fractions. LOX-like activities in S and HMW fractions from chicken breast were much lower than those from beef loin because of the low myoglobin content and high TAC in chicken breast. Reducing compounds such as ascorbic acid can reduce ferrylmyoglobin to metmyoglobin (Giulivi and Cadenas 1993; Kroger-Olsen and Skibsted 1997). Therefore, much higher LOX-like activity in beef loin than chicken breast was attributed to high myoglobin content and low TAC in beef loin.

Lipid oxidation potential of fractions

Lipid oxidation potential (LOP) is the ability to increase lipid oxidation in phospholipid liposome model system during incubation. LOP of each fraction can be determined by the interaction of prooxidant and antioxidant factors in each fraction. Differences in LOP among fractions could be derived from the differences in the balance of prooxidant and antioxidant factors. Metmyoglobin showed very high LOP in phospholipid liposome model systems (data not shown). A significantly higher LOP in H fraction from beef loin compared to that from chicken breast during storage (Figure 3) indicated that there could be a significant difference in the oxidative stability between the 2 meats.

At day 0, the LOP of LMW fraction from chicken breast was the highest, and lipid oxidation by LMW fraction from chicken breast rapidly increased at beginning of incubation, but did not change after 30 min (Figure 3A1). The LOP of LMW fraction from chicken breast at both days 5 and 10 was very low compared to that at day 0. Table 2 showed that FRC of LMW fraction decreased rapidly during storage. Therefore, it is assumed that high FRC at day 0 was attributed to the low-molecular-weight reducing compounds such as ascorbic acid, which reduced the contaminated ferric ion in the reaction solution, leading to the rapid development of lipid oxidation. The LOPs of S and H fraction from chicken breast meat were significantly lower than that of the LMW fraction at day 0, indicating that the HMW and P fraction possessed antioxidant capacity. Kanner and others (1991) indicated that HMW fraction from turkey meat showed an antioxidant effect in model system containing ascorbic acid and ferric ion although LMW fraction acted as a prooxidant in the same system. The difference between LOPs of the H and LMW fractions was significantly greater than that of the S and LMW fractions, due to the higher TAC in the P fraction than that in the HMW fraction (Table 2). At day 10, the LOP of H fraction from chicken breast was significantly lower than those of S, P, and HMW fractions, and was almost the same as that of control (Figure 3A3). Although prooxidant factors in S, P, and HMW fractions, respectively, were present, the interaction of antioxidant capacity between water-soluble (S), especially HMW fraction, and water-insoluble (P)

Table 3 – Lipoxygenase-like activities of various fractions from chicken breast and beef loin during storage at 4 °C.

Storage	Chicken breast			Beef loin			
	S	HMW	LMW	S	HMW	LMW	SEM
	Unit/g meat						
0 d	1.20 ^c	0.19 ^{cy}	0.04 ^{cy}	16.20 ^{bz}	21.26 ^{ay}	0.13 ^{cx}	0.51
5 d	1.30 ^c	0.40 ^{cx}	0.09 ^{oxy}	31.74 ^{by}	34.43 ^{ax}	0.03 ^{cy}	0.41
10 d	1.20 ^b	0.33 ^{bx}	0.14 ^{bx}	37.38 ^{ax}	35.44 ^{ax}	0.03 ^{by}	1.00
SEM	0.06	0.02	0.02	1.34	1.02	0.01	

^{a-c}Means with different superscripts within the same row are significantly different ($P < 0.05$).

^{x-z}Means with different superscripts within the same column are significantly different ($P < 0.05$).

SEM = standard error of the mean. $n = 4$.

Unit/g meat is defined as the amount of enzyme per g meat catalyzing the formation of 1 μmol of hydroperoxide per minute.

S = supernatant fraction; HMW = high molecular weight fraction from supernatant fraction; LMW = low molecular weight fraction from supernatant fraction.

fraction seemed to suppress the prooxidant activities in H fraction from chicken breast.

At day 0, HMW fraction from beef loin showed the highest LOP (Figure 3B1), and the LOP increased during storage (Figure 3B1 to 3B3). Metmyoglobin was most likely responsible for the high LOP of HMW fraction. An increase in metmyoglobin percentage in HMW fraction during storage (Table 1) are closely involved in the increase of the LOP. Myoglobin can be involved in catalyzing and accelerating lipid oxidation via the formation of ferrylmyoglobin or the release of hemein and free ionic iron in the presence of H_2O_2 or lipid hydroperoxides (Kanner and Harel 1985; Harel and others 1988; Kim and Sevanian 1991; Baron and others 2002; Min and Ahn 2005). Ferrylmyoglobin can initiate and propagate the free radical chain reaction of lipid oxidation (Harel and Kanner 1985; Reeder and Wilson 1998; Baron and Andersen 2002). The hemein released from myoglobin can react with lipid hydroperoxides or H_2O_2 to form high oxidation state (Fe^{IV}) of hemein, which can catalyze initiation and propagation of lipid oxidation (Dix and Marrett 1985; Kim and Sevanian 1991). The hemein may be more dangerous because it is more reactive than metmyoglobin and has a hydrophobic characteristic, which enables it to permeate into cell membrane to catalyze lipid oxidation (Kaschnitz and Hatefi 1975; Schmitt and others 1993; Baron and others 2002). Therefore, both ferrylmyoglobin and hemein can contribute to the LOX-like activity of HMW fraction. In addition, free ionic irons released from

myoglobin can be a catalyst for lipid oxidation via the Fenton reaction in the presence of reducing agents (Harel and others 1988; Ahn and Kim 1998; Min and Ahn 2005). The free ionic irons can be responsible for the high LOP of HMW fraction from beef loin because of the FRC of the HMW fraction (Table 2). Therefore, it was suggested that myoglobin could be a major oxidative factor in beef loin. However, it is difficult to say which mechanism is dominant for the high LOP of HMW fraction from beef loin in this study.

LOPs of H and S fraction from beef loin were significantly lower than that of the HMW fraction during storage, due to the TAC of the LMW and P fraction (Table 2). Myoglobin can be stabilized by reducing agents, which can reduce ferrylmyoglobin to metmyoglobin and prevent the disruption of myoglobin to release hemein and/or free ionic iron (Giulivi and Cadenas 1993; Kroger-Ohlson and Skibsted 1997). LMW fraction from beef loin at day 0 showed a similar pattern of increases in TBARS values to that from chicken breast, but its LOP was smaller due probably to its lower FRC at day 0 (Table 2). Consequently, the increase of lipid oxidation in beef loin during storage was caused by the prooxidant factors, probably myoglobin, in HMW fraction. Although the antioxidant activities in P and S (HMW + LMW) fractions lowered the LOP of HMW fraction, those antioxidant activities may be not enough to attenuate the prooxidative activities of myoglobin in HMW fraction from beef loin.

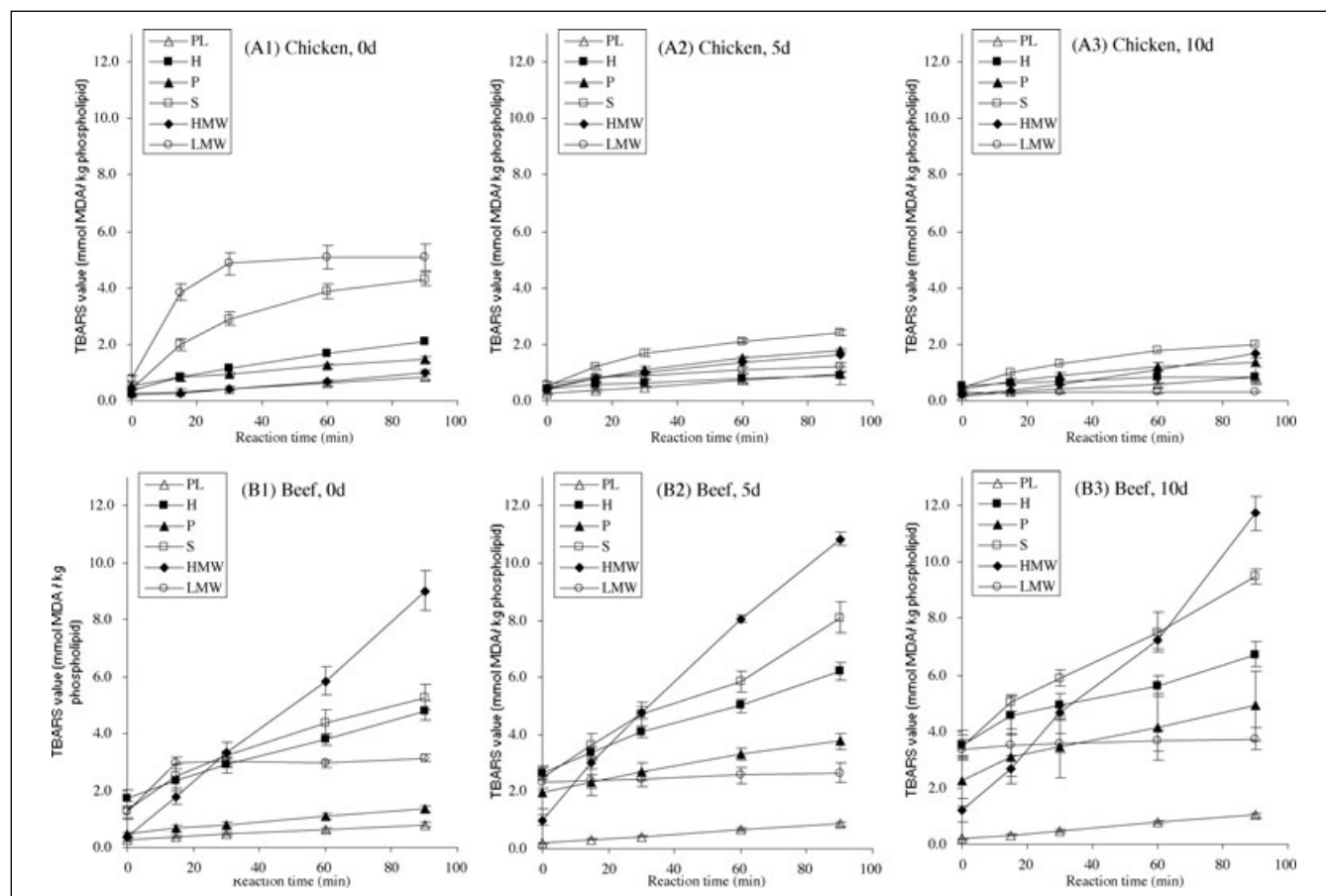


Figure 3—Lipid oxidation potential of various fractions from chicken breast (A1 to A3) and beef loin (B1 to B3) in the phospholipid liposome model system during storage 4 °C during the incubation at 37 °C for 90 min (expressed as TBARS value, mmol malondialdehyde [MDA]/kg phospholipid). The phospholipid liposome model system with 50 mM acetate buffer (pH 5.6) was used as a control (PL). Means with the standard deviation were indicated. $n = 4$. H = homogenate fraction; P = precipitate fraction; S = supernatant fraction; HMW = high molecular weight fraction from supernatant fraction; LMW = low molecular weight fraction from supernatant fraction.

Conclusions

Raw chicken breast was more resistant to oxidative changes than raw beef loin. Higher TAC, lower concentration of myoglobin, and iron chelating ability contributed to the stability of chicken breast. Although the LOP of myoglobin in HMW fraction from beef was decreased by the antioxidant capacity in LMW and P fraction, H fraction from beef loin showed higher LOP than that from chicken breast. Therefore, high myoglobin content, as a source of ferrylmyoglobin, hematin, and free ionic iron, was mainly responsible for the imbalance between endogenous anti- and prooxidant factors, leading to the high susceptibility of beef loin to lipid oxidation. However, it is uncertain which catalytic compound from myoglobin (that is, ferrylmyoglobin, hematin, or free ionic iron) is the predominant factor for the initiation and propagation of lipid oxidation in beef loin.

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